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DISCOVERY

The effect of deteriorative factors on the ascorbic acid level of cashew fruit juice during non-refrigerated storage

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ABSTRACT

Ascorbic acid of cashew apple juice is the major factor used to determine the juice nutrients. It is oxidized easily and the concentration is an index to the retention of the original nutritive quality value. The factors that reduce the ascorbic acid of red and yellow samples of cashew apple juice were estimated and the quality value models based on these factors were developed. It was found that appropriate combination these factors led to satisfactory quality control of ascorbic acid reduction in both samples of cashew fruit juice during non refrigeration storage conditions and the distribution. Results were obtained from 3^4 full factorial experiments conducted in three replicates with the order of the replicates randomized. Multivariate regression analyses were used for relating the variables. The results of the experiments and the models developed for red and yellow samples of cashew fruit juice revealed that pH and duration of storage for red sample and temperature, pH and duration of storage for yellow sample were the major factors that led to reduction of ascorbic acid of the juice and also used in characterization of juice quality. The analysis of variance from both samples of the juice showed that the interactions of total soluble solid with other factors significantly affect the

ascorbic acid of the juice. The red sample had thirty one (31) insignificant regression coefficients while yellow sample had twenty (20) insignificant regression coefficients at 5 percent after checking the adequacy of the predicted models. The positive signs against the coefficients of the main factors and interactions in these models showed that the levels of ascorbic acids were raised by increasing the level of factors from low to intermediate and to high levels while negative signs showed that the levels of ascorbic acids were reduced from low to intermediate and to high levels. The statistical analysis of the experimental data showed that the samples of cashew fruits juice models were adequate for shelf life prediction.

Keywords: Effect, Deteriorative Factors, Ascorbic Acid, Cashew Juice, Refrigerated Storage

1. INTRODUCTION

Losses of vitamins or deterioration of biological values in fruit juices during storage and distribution has little information available as to the rate of these deteriorations. To predict the extent of deterioration of nutrients during storage and distribution of fruit juice, knowledge of the reaction rates as a function of the deteriorative factors is needed (Olorunsogo, 1998).

Different types of fruit juice products are available. These products are preserved in different forms such as pure juice, beverages, squashes, cordials and concentrates. A pure juice is a natural extract from fruits and remains practically unaltered in its composition during its preparation and preservation (Olorunsogo, 1998).

Cashew fruits should be picked from the tree by hand to avoid bruising the delicate flesh. They are then carefully washed and the nuts are removed for processing. Cashew apples should be processed within two to three hours of picking, since they undergo rapid deterioration when kept for a longer time (Hanlon, 2000).

Cashew fruits juice are among the most important foods of mankind and are indispensable for the proper functioning of our bodies and maintenance of our health. They are rich in essential minerals, vitamins, and other nutritive values and are becoming popular on this account. A glass of cashew juice, for example, contains at least a day's supply of vitamin C and often a good deal more. Healthy adults require about 60mg per day of vitamin C (Vaidehi and Ray babu, 2000). Cashew fruit juice has long been noted as excellent sources of ascorbic acid (vitamin C). Ascorbic acid is the least stable of all fruit juice nutrients, as it is readily oxidized (Olorunsogo, 1998).

The wastage of cashew apples is mainly attributed to short shelf life and rapid microbial action. Unlike other fruit juice, the juice extracted from cashew apple cannot be consumed due to its characteristic astringent taste, which causes biting sensation of the tongue and throat. In order to decrease astringency and to prevent spoilage, it is essential to investigate a suitable method for the preservation of cashew apple juice. Various methods of cashew apple juice preservation and shelf life evaluation have been reported by many scientists. Hot fill and aseptic methods were efficient in maintaining physico-chemical characteristics of the juice up to twelve months (Costa et al, 2003). The recommended fruit juice quality were shown in Table 1

Table 1 Recommended Juice Quality

Fruit Juice	**Ascorbic Acid (mg/100ml)**	
	Maximum	**Minimum**
Orange	**80**	**20**
Pineapple	**25**	**8**
Cashew	**510**	**126**
Mango	**80**	**20**
Grape fruit	**65**	**35**
Lemon	**70**	**30**
Lime	**40**	**5**

Source: (Gunjate & Patwardhan, 1995), (Olorunsogo & Adgidzi, 2010)

The physico-chemical analysis of samples of cashew apple juice has revealed the tremendous nutritional potential of this fruit in terms of vitamin C, sugar, organic acids, dry matter and ash. In addition, several compounds with antioxidant capacity such as carotenoids flavonoids, phenolic acids, tannins, and anacardic acids have already been identified. Cashew apple is rich in nutritional composition but is ignored because of its astringency. The identification and determination of organic acids in fruit juice is very important because it provides useful information about the authenticity of the product. Their presence can affect the

chemical and sensory characteristics of these physiochemical parameters like pH, total acidity, microbial stability, softness, overall acceptability and can provide valuable information on product safety and on how to improve some selected technological processes (Chinnici et al., 2005). Cashew apple juice contains thiamine, niacin, riboflavin and precursors of vitamin A. It is also found to be good source of minerals such as copper, zinc, sodium, potassium, calcium, iron, phosphorous and magnesium (Lowor and Agyente-Badu, 2009). In addition to these minerals, the juice also contains sulphur, silicon, chlorine, aluminium and bromine (Marc et al. 2011).

Four main factors have been identified as critical to the retention of ascorbic acid in fruit juice during non-refrigerated storage and distribution. These factors are the storage temperature, the total soluble solid (brix value), the pH and the duration of storage. Balancing these factors will bring about satisfactory control of ascorbic acid degradation in cashew fruit juice during non-refrigerated storage and distribution (Olorunsogo and Adgidzi, 2010). The objective of this work is to estimate the effect of deteriorative factors on the ascorbic acid content of cashew fruit juice during non-refrigerated storage.

2. MATERIALS AND METHOD

Cashew apple fruits samples, comprising of Red and Yellow samples, were obtained from local cashew plantation plot at Ohebe dim in Igbo Etiti Local government of Enugu State, Nigeria. Cashew fruits juice were extracted from cashew apple manually and the obtained juice were filtered using sterilize muslin cloth. The experiments were conducted in Bio Process Laboratory in Agricultural and Bioresource Engineering Department of Enugu State University of Science and Technology, Enugu, Nigeria. The cashew fruit samples and the initial composition of the juices extracted from them are presented in Table 2.

Table 2 Experimental samples

Experimental sample	**Variety/source**	**Properties of juice freshly extracted**		
		Vitamin C	Brix value	pH
Fruit Juice	Red	484.10mg/100ml	11.38^{0}Brix	4.48
Fruit Juice	Yellow	495.65mg/100ml	11.40^{0}Brix	4.60

Experimental Design Method

A four-variable three level factorial experiment provide the framework for designing the juice multifactor experiments. With four variables three levels, a complete design leads to a total of 81 runs. In the 3^4 full factorial experiment the low, intermediate and high levels of the factors are coded as "-", "0"and "+", respectively. The levels of the four factors which include temperature, total soluble solid, pH and duration of storage are represented in standard order as x_1, x_2, x_3 and x_4.

Conduct of Experiment

Four variable three level factorial experiments were conducted in a randomized order in three replicates according to the design plan (matrix) given in Table 3. The plus, zero and minus signs in the columns indicate how to combine the factors in each experimental run. For example, the first run puts all the four factors at their low levels, the second run sets factors x_1 at high level while all the other factors will be keep at intermediate and low levels. The coded levels of the factors and the results of each sample experiments are given in Table 4

Table 3 Design Matrix for 3^4 Full Factorial Experiment

Run	x_0	x_1	x_2	x_3	x_4	x_1x_2	x_1x_3	x_1x_4	x_2x_3	x_2x_4	x_3x_4	$x_1x_2x_3$	$x_1x_2x_4$	$x_1x_3x_4$	$x_2x_3x_4$	$x_1x_2x_3x_4$
1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1
2	+1	0	+1	+1	+1	0	0	0	+1	+1	+1	0	0	0	+1	0
3	+1	-1	+1	+1	+1	-1	-1	-1	+1	+1	+1	-1	-1	-1	+1	-1
4	+1	+1	0	+1	+1	0	+1	+1	0	0	+1	0	0	+1	0	0
5	+1	0	0	+1	+1	-2	0	0	0	0	+1	-2	-2	0	0	-2
6	+1	-1	0	+1	+1	0	-1	-1	0	0	+1	0	0	-1	0	0
7	+1	+1	-1	+1	+1	-1	+1	+1	-1	-1	+1	-1	-1	+1	-1	-1
8	+1	0	-1	+1	+1	0	0	0	-1	-1	+1	0	0	0	-1	0
9	+1	-1	-1	+1	+1	+1	-1	-1	-1	-1	+1	+1	+1	-1	-1	+1
10	+1	+1	+1	0	+1	+1	0	+1	0	+1	0	0	+1	0	0	0

11	+1	0	+1	0	+1	0	-2	0	0	+1	0	-2	0	-2	0	-2
12	+1	-1	+1	0	+1	-1	0	-1	0	+1	0	0	-1	0	0	0
13	+1	+1	0	0	+1	0	0	+1	-2	0	0	-2	0	0	-2	-2
14	+1	0	0	0	+1	-2	-2	0	-2	0	0	0	-2	-2	-2	0
15	+1	-1	0	0	+1	0	0	-1	-2	0	0	-2	0	0	-2	-2
16	+1	+1	-1	0	+1	-1	0	+1	0	-1	0	0	-1	0	0	0
17	+1	0	-1	0	+1	0	-2	0	0	-1	0	-2	0	-2	0	-2
18	+1	-1	-1	0	+1	+1	0	-1	0	-1	0	0	+1	0	0	0
19	+1	+1	+1	-1	+1	+1	-1	+1	-1	+1	-1	-1	+1	-1	-1	-1
20	+1	0	+1	-1	+1	0	0	0	-1	+1	-1	0	0	0	-1	0
21	+1	-1	+1	-1	+1	-1	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1
22	+1	+1	0	-1	+1	0	-1	+1	0	0	-1	0	0	-1	0	0
23	+1	0	0	-1	+1	-2	0	0	0	0	-1	+2	-2	0	0	+2
24	+1	-1	0	-1	+1	0	+1	-1	0	0	-1	0	0	+1	0	0
25	+1	+1	-1	-1	+1	-1	-1	+1	+1	-1	-1	+1	-1	-1	+1	+1
26	+1	0	-1	-1	+1	0	0	0	+1	-1	-1	0	0	0	+1	0
27	+1	-1	-1	-1	+1	+1	+1	-1	+1	-1	-1	-1	+1	+1	+1	-1
28	+1	+1	+1	+1	0	+1	+1	0	+1	0	0	+1	0	0	0	0
29	+1	0	+1	+1	0	0	0	-2	+1	0	0	0	-2	-2	0	-2
30	+1	-1	+1	+1	0	-1	-1	0	+1	0	0	-1	0	0	0	0
31	+1	+1	0	+1	0	0	+1	0	0	-2	0	0	-2	0	-2	-2
32	+1	0	0	+1	0	-2	0	-2	0	-2	0	-2	0	-2	-2	0
33	+1	-1	0	+1	0	0	-1	0	0	-2	0	-2	-2	0	-2	-2
34	+1	+1	-1	+1	0	-1	+1	0	-1	0	0	-1	0	0	0	0
35	+1	0	-1	+1	0	0	0	-2	-1	0	0	+1	-2	-2	0	-2
36	+1	-1	-1	+1	0	+1	-1	+1	-1	0	0	+1	0	0	0	0
37	+1	+1	+1	0	0	+1	0	0	0	0	-2	0	0	-2	-2	-2
38	+1	0	+1	0	0	0	-2	-2	0	0	-2	-2	-2	0	-2	0
39	+1	-1	+1	0	0	-1	0	0	0	0	-2	0	0	-2	-2	+2
40	+1	+1	0	0	0	0	0	0	-2	-2	-2	-2	-2	-2	0	0
41	+1	0	0	0	0	-2	-2	-2	-2	-2	-2	0	0	0	0	-2

Run	x_0	x_1	x_2	x_3	x_4	x_1x_2	x_1x_3	x_1x_4	x_2x_3	x_2x_4	x_3x_4	$x_1x_2x_3$	$x_1x_2x_4$	$x_1x_3x_4$	$x_2x_3x_4$	$x_1x_2x_3x_4$
42	+1	-1	0	0	0	0	0	0	-2	-2	-2	-2	-2	-2	0	0
43	+1	+1	-1	0	0	-1	0	0	0	0	-2	0	0	-2	-2	-2
44	+1	0	-1	0	0	0	-2	-2	0	0	-2	-2	-2	0	-2	0
45	+1	-1	-1	0	0	+1	0	0	0	0	-2	0	0	-2	-2	-2
46	+1	+1	+1	-1	0	+1	-1	0	-1	0	0	-1	0	0	0	0
47	+1	0	+1	-1	0	+1	0	-2	-1	0	0	-1	-2	-2	0	-2
48	+1	-1	+1	-1	0	-1	+1	0	-1	0	0	+1	0	0	0	0
49	+1	+1	0	-1	0	0	-1	0	0	-2	0	0	-2	0	-2	-2
50	+1	0	0	-1	0	-2	0	-2	0	-2	0	+2	0	-2	-2	0
51	+1	-1	0	-1	0	0	+1	0	0	-2	0	0	-2	0	-2	-2
52	+1	+1	-1	-1	0	-1	-1	0	+1	0	0	+1	0	0	0	0
53	+1	0	-1	-1	0	0	0	-2	+1	0	0	0	-2	-2	0	-2
54	+1	-1	-1	-1	0	+1	+1	0	+1	0	0	-1	0	0	0	0
55	+1	+1	+1	+1	-1	+1	+1	-1	+1	-1	-1	+1	-1	-1	-1	-1
-56	+1	0	+1	+1	-1	0	0	0	+1	-1	-1	0	0	0	-1	0
57	+1	-1	+1	+1	-1	-1	-1	+1	+1	-1	-1	-1	+1	+1	-1	+1
58	+1	+1	0	+1	-1	0	+1	-1	0	0	-1	0	0	-1	0	0
59	+1	0	0	+1	-1	-2	0	0	0	0	-1	-2	+2	0	0	+2
60	+1	-1	0	+1	-1	0	-1	+1	0	0	-1	0	0	+1	0	0
61	+1	+1	-1	+1	-1	-1	+1	-1	-1	+1	-1	-1	+1	-1	+1	+1
62	+1	0	-1	+1	-1	0	0	0	-1	+1	-1	0	0	0	+1	0
63	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1	-1	+1	-1	+1	+1	-1
64	+1	+1	+1	0	-1	+1	0	-1	0	-1	0	0	-1	0	0	0
65	+1	0	+1	0	-1	0	-2	0	0	-1	0	-2	0	+2	0	+2

66	+1	-1	+1	0	-1	-1	0	+1	0	-1	0	0	+1	0	0	0
67	+1	+1	0	0	-1	0	0	-1	-2	0	0	-2	0	0	+2	+2
68	+1	0	0	0	-1	-2	-2	0	-2	0	0	0	+2	+2	+2	0
69	+1	-1	0	0	-1	0	0	+1	-2	0	0	-2	0	0	+2	+2
70	+1	+1	-1	0	-1	-1	0	-1	0	+1	0	0	+1	0	0	0
71	+1	0	-1	0	-1	0	-2	0	0	+1	0	-2	0	+2	0	+2
72	+1	-1	-1	0	-1	+1	0	+1	0	+1	0	0	-1	0	0	0
73	+1	+1	+1	-1	-1	+1	-1	-1	-1	-1	+1	-1	-1	+1	+1	+1
74	+1	0	+1	-1	-1	0	0	0	-1	-1	+1	0	0	0	+1	0
75	+1	-1	+1	-1	-1	-1	+1	+1	-1	-1	+1	+1	+1	-1	+1	+1
76	+1	+1	0	-1	-1	0	-1	-1	0	0	+1	0	0	+1	0	0
77	+1	0	0	-1	-1	-2	0	0	0	0	+1	+2	+2	0	0	-2
78	+1	-1	0	-1	-1	0	+1	+1	0	0	+1	0	0	-1	0	0
79	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	-1	-1
80	+1	0	-1	-1	-1	0	0	0	+1	+1	+1	0	0	0	-1	0
81	+1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	+1

Statistical Analysis and Model Development

Table 4 Factors and their Coded Levels

Level of Factors	**Code**	**Juice Sample**	**Independent variables**			
			Temperate (x_1)	Total soluble solid (x_2)	pH (x_3)	Duration of storage (x_4)
Based level	x	Red	34.15^{0}C	10.31^{0}Brix	3.91	11days
		Yellow	34.15^{0}C	10.64^{0}Brix	3.86	11days
Interval of Variation	ΔXi	Red	4.45^{0}C	0.82^{0}Brix	0.60	5days
		Yellow	4.45^{0}C	1.04^{0}Brix	0.75	5days
High level	+	Red	38.60^{0}C	11.13^{0}Brix	4.51	16days
		Yellow	38.60^{0}C	11.68^{0}Brix	4.61	16days
Intermediate	0	Red	34.40^{0}C	10.56^{0}Brix	3.99	11days
		Yellow	34.40^{0}C	10.59^{0}Brix	3.98	11days
Low level	-	Red	29.70^{0}C	9.50^{0}Brix	3.32	6days
		Yellow	29.70^{0}C	9.61^{0}Brix	3.12	6days

Multivariate regression analysis was used in relating the variables. The mean of the replicated observations were given by

The mean, $\bar{y}_u = \frac{1}{r}\sum_{v=1}^{r} y_{uv} i$ r = replicate 1

The dispersion, $S_u^2 = \frac{1}{r-1}\sum_{v=1}^{r}(y_{uv} - \bar{y}_u)^2$ 2

The sum of the dispersion $\sum_{u=1}^{81} S_u^2$ 3

The maximum dispersion = $S_{u\max}^2$ 4

Where

r = replication, y_{uv} = value of each ascorbic acid measure, $\bar{y}_u$ = mean of the experimental observation, S_u^2 = dispersion

The G-test (Cochran G-criteria) is used to ascertain the possibility of carrying out regression analysis. It is used to check if the output factors of the replication have maximum accuracy of the replication. The test verifies the homogeneity of dispersion of the replicate experiments. The calculated G-value is given as:

$$G_{cal} = \frac{S^2_{u\max}}{\sum_{u=1}^{N} S_u^2}; N = 81 \qquad 5$$

The calculated G-value is compared with an appropriate table value. The condition of homogeneity is given as:

$$G_{cal} < G_{[\alpha, N, (r-1)]}. \qquad 6$$

where, N = Number of experimental runs , r = Number of replicate, α = Level of significance

The dispersion, taken as mean-squared-error, is given as:

$$S^2_{(y)} = \frac{1}{N}\sum_{u=1}^{N} S_u^2. \qquad 7$$

It is the average sample variance estimate. The experimental error is given as:

$$S_{(y)} = \sqrt{S^2_{(y)}} \qquad 8$$

The mean effect was estimated by

$$b_0 = \frac{1}{N}\sum_{u=1}^{N}\left(x_0\, \bar{y}_u\right); u = 1,2,\ldots\ldots\ldots\ldots\ldots\ldots,81 \qquad 9$$

where x_0 was the coded signs in the x_0 column of the design matrix.

The four main effects were estimated by

$$b_i = \frac{1}{N}\sum_{u=1}^{N}\left(x_i\, \bar{y}_u\right); i = 1,2,\ldots\ldots\ldots\ldots\ldots\ldots,4; \qquad 10$$

where x_i were the coded signs in the x_l columns of the design matrix.

The six two-factor interactions were estimated by

$$b_{ij}\ \frac{1}{N}\sum_{u=1}^{N}\left(x_{ij}\, \bar{y}_u\right); i \neq j;; u = 1,2,\ldots\ldots\ldots\ldots\ldots\ldots,81 \qquad 11$$

where x_{ij} were the coded signs in the x_{ij} columns of the design matrix.

the four three-factor interactions were estimated by

$$b_{ijkl} = \frac{1}{N}\sum_{u=1}^{N}\left(x_{ijkl}\, \bar{y}_u\right); i \neq j \neq k; \quad ; \quad \mathrm{u} = 1,2,\ldots\ldots\ldots\ldots\ldots\ldots,81 \qquad 12$$

where x_{ijkl} were the coded signs in the x_{ijkl} columns of the design matrix.

The one four-factor interactions were estimated by

$$b_{ijkl} = \frac{1}{N}\sum_{u=1}^{N}\left(x_{ijkl}\, \bar{y}_u\right); i \neq j \neq k \neq l; u = 1,2,\ldots\ldots\ldots\ldots\ldots\ldots,81 \qquad 13$$

where x_{ijkl} were the coded signs in the x_{ijkl} columns of the design matrix

Construction of confidence interval and testing of hypotheses about individual regression coefficients in the regression model are frequently used in assessing their statistical significance (Samprit and Bertram, 1991).

Confidence interval for the regression coefficients with confidence coefficient "α" was of the general form.

$b's \pm t\{\alpha, N(r-1\} S_{b's}$

i.e $b's \pm \Delta b's$ 14

where, $S_{b's}$ = the estimated standard error in regression coefficients b's.

$t\{\alpha, N(r-1\}$ = are appropriate tabulated criteria with

N(r-1) degree of freedom

For our purpose, we were contented with a level of significance of 5% (i.e α = 0.05), with this we established confidence limits for 99% of the variable measurements, using a 95% confidence interval. That was, approximately 95 out of every 100 similarly constructed confidence intervals will contain 99% of the variable measurements in the population.

For full factorial experiments, errors in each regression coefficient is the same and was determined by

$$S_{bo} = Sb_i \ldots\ldots\ldots\ldots\ldots Sb_{ijklm} = \frac{S(r)}{\sqrt{Nr}}. \quad 15$$

where $S_{bi}^2 = \frac{S_y^2}{N}$ 16

where S(y) = the experimental error. The statistical significance of the regression coefficients were tested by

$$t_0 = \frac{b_0}{S_{b0}}, \quad t_i = \frac{b_i}{S_{bi}}, \; t_w = \frac{b_{ij}}{S_{bij}} \;\bullet\bullet\bullet\bullet\bullet\bullet\; t_{ijklm} = \frac{\{b_{ijklm}\}}{S_{bijklm}} \quad 17$$

The test was carried out by comparing these calculated t-values with the appropriate critical table values. A coefficient of regression is statically significant if and only if

$t_{cal} > t\{\alpha, N(r-1)\}$ 18

if any coefficient is statistically insignificant (i.e $t_{cal} < t_{table}$), such a coefficient is left out of the regression model (Douglas, 1991). Insignificance of an effect does not necessarily mean that the particular factors or interaction is unimportant. It only implies that response is unaffected if the factor is varied over the range considered (i.e. from -1 to +1or 0 in coded units).

The sums of squares for the effects were computed from the contrasts used in estimating the effects. In the 3^k factorial design with replicates, the regression sum of squares for any effects were computed with equation 19.

$$SS_R = \frac{r}{N}(contrast)^2 \quad 19$$

and has a single degree of freedom. Consequently, the main effects and the interactions were computed using equations 21 to 24.

$$SS_{bi} = \frac{r}{N}\sum_{u=1}^{N}\left(x_i \bar{Y}_u\right)^2 \quad 20$$

where x_i were the coded signs in the x_i column of the design matrix.

For the two-factor interactions

$$SS_{bij} = \frac{r}{N}\sum_{u=1}^{N}\left(x_{ij} \bar{Y}_u\right)^2; i = \quad 21$$

where x_{ij} were the coded signs in the x_{ij} column of the design matrix.

For the three-factor interactions

$$SS_{bijk} = \frac{r}{N}\sum_{u=1}^{N}\left(x_{ijk}\,\bar{Y}_u\right)^2 ;i\ \ j\ \ k \quad 22$$

where x_{ijk} were the coded signs in the x_{ijk} columns of the design matrix

For the four-factor interactions

$$SS_{bijkl} = \frac{r}{N}\sum_{u=1}^{N}\left(x_{ijkl}\,\bar{Y}_u\right)^2 ;i\ \ j\ \ k\ \ l \quad 23$$

where x_{ijkl} were the coded signs in the x_{ijkl} columns of the design matrix.

note that $N = 3^k$.

The total sum of squares was found by

$$SS_T = \sum_{u=1}^{N.r} Y^2uv - \sum_{u=1}^{N.r}(Yuv)^2 / N.r \quad 24$$

The error sum of squares was given as;

$$SS_E = SS_T = -\sum SS_R \quad 25$$

$$i.e\ SS_E = SS_T - SS_{bj.} + \ldots\ldots. + SS_{bij} + \ldots\ldots\ldots\ldots\ldots\ldots + SS_{bijklm} \quad \text{(Douglas, 1991)} \quad 26$$

In multiple linear regressions, testing the significance or contribution of individual coefficient is accomplished by testing the null hypothesis H_0: $b_i = 0$. The appropriate statistics for the F-test is

$$F_{cal} = \frac{MS_R}{MS_E} = \frac{SS_R / df_R}{SS_E / N(r-1)} \quad 27$$

Where df_R = the degree of freedom regression

The null hypothesis will be rejected if

$$F_{cal} > F\{\alpha, df_R, N(r-1)\} \quad 28$$

With the conclusion that the coefficient contributes significantly to the regression (Douglas, 1991). The complete analyses of variance were summarized using the conclusion. The adequacy of the model was further checked. A method of validating the model adequacy is to calculate the dispersion of adequacy for the replicate experiment and compared the magnitude with the variance estimate given by the mean squared error. The dispersion of adequacy for the replicate experiment is given

$$SS^2_{(ad)} = \frac{r}{N-\lambda}\sum_{u=1}^{N}\left(\bar{y}_u - \hat{y}_u\right)^2 = \frac{r}{df_{ad}}\sum_{u=1}^{N}\left(\bar{y}_u - \hat{y}_u\right)^2 \quad 29$$

where λ = number of inadequate coefficients.

The adequacy of the regression model was estimated by Fisher's criteria (F-test).

$$F_{cal} = \frac{S_{(ad)}}{S^2_{(y)}} \quad 30$$

Where $S^2_{(y)}$ = variance estimate given by the mean squared error. The calculated F-value was compared with the appropriate table value. The condition of adequacy is

$$F_{cal} \le F\{\alpha, N-\lambda, N(r-1)\} \quad 31$$

If this condition is satisfied then we can conclude that the fitted (or predicted) regression model is adequate.

3. RESULTS AND DISCUSSION

The data generated, which consists of the 81 runs that were replicated of three observations of the dependent variable 'y' of red and yellow cashew fruits juice samples were used to produced the models and the analysis of variance presented in Tables 5and 6.

The adequacies of the predicted models were evaluated by testing hypothesis on the individual regression coefficients. The sum of squares for the effects and total sum of squares for both samples were estimated using equations 19 through 23. The complete analyses of variance for red and yellow samples were summarized in Tables 5 and 6 respectively.

Table 5 Analysis of Variance for Replicated 3^4 Factorial Experiment for Red Sample of Cashew Fruit Juice

Source of variation	Effect	Sum of squares	Degree freedom	Means squares	Fraction
b_1	5.39	7055.78	1	7055.78	1.625
b_2	2.56	1590.53	1	1590.53	0.366
b_3	-13.41	43,677.31	1	43677.31	10.062
b_4	-15.76	60,331.17	1	60,331.17	13.899
b_{12}	-33.21	26,8055.51	1	268,055.51	61.754
b_{13}	-40.50	39,8639.07	1	398,639.07	91.838
b_{14}	-45.65	506,294.57	1	506,294.57	116.639
b_{23}	-25.47	157,663.63	1	157,667.63	36.322
b_{24}	-30.44	225,133.02	1	225,133.02	51.866
b_{34}	-22.04	118,025.52	1	118,025.52	27.190
b_{123}	-55.49	748,287,65	1	748,287.65	172.389
b_{124}	-42.08	430,283.99	1	430,283.99	99.128
b_{134}	-42.96	448,47.65	1	448,427.65	103.308
b_{234}	-55.39	745,619.75	1	745,619.75	171.774
b_{1234}	-43.57	461,274.28	1	461,274.28	106.267
b_1^2	4.03	3,948.47	1	3,948.47	0.910
b_2^2	5.66	7,791.10	1	7,791.10	1.795
b_3^2	11.13	30,125.46	1	30,125.46	6.940
b_4^2	27.68	186,139.14	1	186,139.14	42.882
$b_1^2 b_2$	-0.84	172.52	1	172.52	0.040
$b_1^2 b_3$	3.25	2,574.49	1	2574.49	0.593
$b_1^2 b_4$	1.57	600.38	1	600.38	0.138
$b_1^2 b_{23}$	-15.41	57,689.03	1	57,689.03	13.290
$b_1^2 b_{24}$	-8.87	19,106.78	1	19,106.78	4.402
$b_1^2 b_{34}$	1.20	347.26	1	347.26	0.080
$b_1^2 b_{234}$	-9.41	21,496.30	1	21,496.30	4.952

$b_2^2 b_1$	-4.55	5,028.25	1	5,028.25	1.158
$b_2^2 b_3$	-0.49	59.17	1	59.17	0.014
$b_2^2 b_4$	7.37	13,203.87	1	13,203.87	3.042
$b_2^2 b_{13}$	-10.43	26,437.23	1	26,437.23	6.091
$b_2^2 b_{14}$	-5.02	6,135.75	1	6,135.75	1.414
$b_2^2 b_{34}$	-4.07	4,019.17	1	4,019.17	0.926
$b_2^2 b_{134}$	1.61	631.33	1	631.33	0.145
$b_3^2 b_1$	-10.67	27,688.33	1	27,688.33	6.379
$b_3^2 b_2$	-9.81	23,385.37	1	23,385.37	5.387
$b_3^2 b_4$	-16.74	68,076.22	1	68,076.22	15.683
$b_3^2 b_{12}$	2.70	1769.04	1	1769.04	0.408
$b_3^2 b_{14}$	-19.53	92,641.93	1	92,641.93	21.343
$b_3^2 b_{24}$	-0.41	40.24	1	40.24	0.009
$b_3^2 b_{124}$	0.76	142.28	1	142.28	0.033
$b_4^2 b_1$	-6.75	11,075.33	1	11,075.33	2.552
$b_4^2 b_2$	17.22	72,056.49	1	72,056.49	16.600
$b_4^2 b_3$	9.68	22,763.24	1	22,763.24	5.244
$b_4^2 b_{12}$	-9.52	22,026.04	1	22,026.04	5.074
$b_4^2 b_{13}$	10.79	28,279.40	1	28,279.40	6.515
$b_4^2 b_{23}$	-3.37	2,751.85	1	2,751.85	0.634
$b_4^2 b_{123}$	-17.25	72,333.56	1	72,333.56	16.664
$b_1^2 b_2^2$	-31.73	244,589.76	1	244,589.76	56.348
$b_1^2 b_3^2$	10.88	28,756.49	1	28,756.49	6.625
$b_1^2 b_4^2$	-1.43	495.45	1	495.45	0.114
$b_2^2 b_3^2$	-0.90	198.07	1	198.07	0.046
$b_2^2 b_4^2$	3.99	3,868.82	1	3,868.82	0.891
$b_3^2 b_4^2$	33.33	270,026.00	1	270,026.00	62.208
$b_1^2 b_2^2 b_3^2$	-6.14	9,169.85	1	9,169.85	2.113
$b_1^2 b_2^2 b_4^2$	8.46	17,375.15	1	17,375.15	4.003
$b_1^2 b_3^2 b_4^2$	16.54	66,496.76	1	66,496.76	15.319
$b_2^2 b_3^2 b_4^2$	-6.77	11,123.99	1	11,123.99	2.563
$b_1^2 b_2^2 b_3^2 b_4^2$	0.62	94.90	1	94.90	0.022
$b_1^2 b_2^2 b_3$	8.34	16,897.51	1	16,897.51	3.893

$b_1^2b_2^2b_4$	8.50	17,552.16	1	17,552.16	4.044
$b_1^2b_2^2b_{34}$	11.49	32,069.17	1	32,069.17	7.388
$b_1^2b_3^2b_2$	4.32	4,527.97	1	4,527.97	1.043
$b_1^2b_3^2b_4$	10.85	28,602.64	1	28,602.64	6.589
$b_1^2b_3^2b_{24}$	-17.03	70,492.45	1	70,492.45	16.240
$b_1^2b_4^2b_2$	-3.04	2,247.72	1	2,247.72	0.518
$b_1^2b_4^2b_3$	-18.34	82,127.73	1	82,127.73	18.920
$b_1^2b_4^2b_{23}$	-14.69	52,412.89	1	52,412.89	12.075
$b_2^2b_3^2b_1$	11.22	30,574.05	1	30,574.05	7.044
$b_2^2b_3^2b_4$	39.61	381,262.49	1	381,262.49	87.834
$b_2^2b_3^2b_{14}$	0.97	230.04	1	230.04	0.053
$b_2^2b_4^2b_1$	-9.76	23,134.72	1	23,134.72	5.330
$b_2^2b_4^2b_3$	-21.86	116,131.69	1	116,131.69	26.754
$b_2^2b_4^2b_{13}$	-14.02	47,791.94	1	47,791.94	11.010
$b_3^2b_4^2b_1$	6.31	9,679.48	1	9,679.48	2.230
$b_3^2b_4^2b_2$	-8.87	19,101.99	1	19,101.99	4.401
$b_3^2b_4^2b_{12}$	-6.25	9,501.19	1	9,501.19	2.189
$b_1^2b_2^2b_3^2b_4$	16.23	64,019.07	1	64,019.07	14.749
$b_1^2b_2^2b_4^2b_3$	-3.81	3,525.36	1	3,525.36	0.812
$b_1^2b_3^2b_4^2b_2$	39.09	371,369.47	1	371,369.47	85.555
$b_2^2b_3^2b_4^2b_1$	-8.37	17,040.91	1	17,040.91	3.926
Error Total		703,192.92 8,206,575.47	162 242	4340.70	

Table 6 Analysis of Variance for Replicated 3^4 Factorial Experiment for Yellow Sample of Cashew Fruit Juice

Source of variation	Effect	Sum of squares	Degree freedom	Means squares	Fraction
b_1	7.08	12,188.79	1	12,188.79	7.563
b_2	4.45	4,806.14	1	4,806.14	2.982
b_3	-11.76	33,694.57	1	33,694.57	20.907
b_4	-11.45	31,848.98	1	31,848.98	19.762
b_{12}	-32.95	263,790.12	1	263,790.12	163.679
b_{13}	-36.07	316,233.99	1	316,233.99	196.220
b_{14}	-16.29	64,494.23	1	64,494.23	40.018
b_{23}	-27.78	187,573.33	1	187,573.33	116.387

b_{24}	-32.73	260,273.33	1	260,273.33	161.497
b_{34}	-17.80	77,025.32	1	77,025.32	47.793
b_{123}	-53.20	687,786.62	1	687,786.62	426.765
b_{124}	-41.60	420,620.93	1	420,620.93	260.991
b_{134}	-40.01	388,939.21	1	388,939.21	241.333
b_{234}	-54.45	720,427.41	1	120,427.91	74.724
b_{1234}	-40.52	398,938.04	1	398,938.04	247.537
b_1^2	6.85	11,398.88	1	11,398.88	7.073
b_2^2	18.47	82,897.24	1	82,897.24	51.437
b_3^2	27.70	186,451.47	1	186,451.47	115.691
b_4^2	55.30	743,115.87	1	743,115.87	461.096
$b_1^2 b_2$	4.62	5,179.48	1	5,179.48	3.214
$b_1^2 b_3$	0.04	0.454	1	0.454	0.0003
$b_1^2 b_4$	-0.17	6.82	1	6.82	0.004
$b_4^2 b_{13}$	-597	8,658.23	1	8,658.23	5.372
$b_4^2 b_{23}$	-3.65	3,233.65	1	3,233.65	2.006
$b_4^2 b_{123}$	-17.11	71,11.04	1	71,11.04	44.124
$b_1^2 b_2^2$	59.96	873,673.66	1	873,673.66	542.106
$b_1^2 b_3^2$	4.20	4,294.34	1	4,294.34	2.665
$b_1^2 b_4^2$	-26.64	172,470.56	1	172,470.56	107.016
$b_2^2 b_3^2$	-13.91	46,989.41	1	46,989.41	29.162
$b_2^2 b_4^2$	8.31	16,780.63	1	16,780.63	10.412
$b_3^2 b_4^2$	0.20	9.88	1	9.88	0.006
$b_1^2 b_2^2 b_3^2$	-28.90	202,912.68	1	202,912.68	125.91
$b_1^2 b_2^2 b_4^2$	8.41	17,176.33	1	17,176.33	10.658
$b_1^2 b_3^2 b_4^2$	15.21	56,204.75	1	56,204.75	34.874
$b_2^2 b_3^2 b_4^2$	13.28	42,869.43	1	42,869.43	26.600
$b_1^2 b_2^2 b_3^2 b_4^2$	-4.42	4,754.24	1	4,754.24	2.950
$b_1^2 b_2^2 b_3^2$	24.94	151091.51	1	151091.51	93.750
$b_1^2 b_2^2 b_4$	-1.00	242.40	1	242.40	0.150
$b_1^2 b_2^2 b_{34}$	8.15	80,089.97	1	80,089.97	49.695
$b_1^2 b_3^2 b_2$	-5.47	7,268.81	1	7,268.81	4.510
$b_1^2 b_3^2 b_4$	7.06	12,124.71	1	12,124.71	7.523
$b_1^2 b_3^2 b_{24}$	-23.47	133,867.00	1	133,867.00	83.063

$b_1^2 b_4^2 b_2$	-3.30	2,653.80	1	2,653.80	1.647
$b_1^2 b_4^2 b_3$	23.67	136,116.94	1	136,116.94	84.459
$b_2^2 b_4^2 b_{13}$	29.57	211,608.83	1	211,608.83	131.301
$b_2^2 b_3^2 b_1$	-2.71	1,782.99	1	1,782.99	1.106
$b_2^2 b_3^2 b_4$	27.94	189,696.39	1	189,696.39	117.705
$b_2^3 b_3^2 b_{14}$	-0.95	220.28	1	220.28	0.137
$b_2^2 b_4^2 b_1$	-18.35	81781.73	1	81781.73	50.745
$b_2^2 b_4^2 b_3$	-26.95	176,548.11	1	176,548.11	109.546
$b_2^2 b_4^2 b_{13}$	-13.98	47,473.57	1	47,473.57	29.457
$b_3^2 b_4^2 b_1$	13.00	41,050.62	1	41,050.62	25.471
$b_3^2 b_4^2 b_2$	-11.35	31,277.99	1	31,277.99	19.408
$b_3^2 b_4^2 b_{12}$	0.99	237.69	1	237.69	0.147
$b_1^2 b_2^2 b_{34}$	6.26	9,831.16	1	9,831.16	6.100
$b_1^2 b_2^2 b_4^2 b_3$	-4.68	5,317.23	1	5,317.23	3.299
$b_1^2 b_3^2 b_4^2 b_2$	32.92	263,278.36	1	263,278.36	163.362
$b_2^2 b_3^2 b_4^2 b_1$	-6.25	9,500.44	1	9,500.44	5.895
Error Total		261,084.67 8,781,259.62	162 242	1,611.63	

Considering only the significant coefficient, the fitted or predicted models for red (equation 32) and yellow (equation 33) samples become. The calculations of equations 32 and 33 at the levels of the independent variables provide the fitted values. Using the predicted models, the predicted values of 'y' at the eighty-one points in the design were generated for both samples. The adequacy of the predicted model was checked by calculating the dispersion of adequacy and comparing the magnitude with the variance estimate given by the mean squared error.

$$\begin{aligned}
y_u &= 207.11 - 15.96x_3 - 18.76x_4 - 39.54x_{12} - 48.21x_{13} - 54.35x_{14} - 30.32x_{23} - 36.24x_{24} - 26.24x_{34} \\
&- 66.06x_{123} - 50.10x_{124} - 51.14x_{134} - 65.94x_{234} - 51.87x_{1234} + 13.25x_3^2 + 32.95x_4^2 - 18.35x_1^2x_{23} + \\
&10.56x_1^2x_{24} - 11.20x_1^2x_{234} - 12.42x_2^2x_{13} - 12.7x_3^2x_1 - 11.68x_3^2x_2 - 19.93x_3^2x_4 - 23.25x_3^2x_{14} + 20.5x_4^2x_2 \\
&+ 11.52x_4^2x_3 - 11.33x_4^2x_{12} + 12.85x_4^2x_{13} - 20.54x_4^2x_{123} - 37.77x_1^2x_2^2 + 12.95x_1^2x_3^2 + 39.68x_3^2x_4^2 + 10.07x_1^2x_2^2x_4^2 \\
&+ 19.69x_1^2x_3^2x_4^2 + 9.93x_1^2x_2^2x_3 + 10.12x_1^2x_2^2x_4 + 13.68x_1^2x_2^2x_{34} + 12.92x_1^2x_3^2x_4 - 20.27x_1^2x_3^2x_{24} - 21.83x_1^2x_4^2x_3 \\
&- 17.49x_1^2x_4^2x_{23} + 13.36x_2^2x_3^2x_1 + 47.15x_2^2x_3^2x_4 - 11.62x_2^2x_4^2x_1 - 26.02x_2^2x_4^2x_3 - 16.29x_2^2x_4^2x_{13} - 10.56x_3^2x_4^2x_2 \\
&+ 19.32x_1^2x_2^2x_3^2x_4 + 46.54x_1^2x_3^2x_4^2x_2 - 9.96x_2^2x_3^2x_4^2x_1 \qquad 32
\end{aligned}$$

$$
\begin{aligned}
y_u &= 240.07 + 12.87x_1 - 21.38x_3 - 20.82x_4 - 59.91x_{12} - 65.58x_{13} - 29.62x_{14} - 50.51x_{23} - 59.51x_{24} \\
&- 32.36x_{34} - 96.73x_{123} - 75.64x_{124} - 72.75x_{134} - 99.0x_{234} - 73.67x_{1234} + 12.45x_1^2 + 33.58x_2^2 + 50.36 \\
&x_3^2 + 100.55x_4^2 - 23.58x_1^2x_{23} - 21.71x_1^2x_{24} - 16.75x_1^2x_{234} - 9.44x_2^2x_1 + 10.95x_2^2x_3 + 29.62x_2^2x_4 - 14.45x_2^2x_{13} \\
&- 12.29x_2^2x_{14} + 20.75x_2^2x_{134} - 9.47x_3^2x_1 - 9.95x_3^2x_2 - 14.98x_3^2x_{14} - 9.91x_3^2x_{24} + 28.6x_4^2x_2 + 37.20x_4^2x_3 - \\
&29.69x_4^2x_{12} - 10.85x_4^2x_{13} - 31.11x_4^2x_{123} + 10.9x_1^2x_2^2 - 48.44x_1^2x_4^2 - 25.29x_2^2x_3^2 + 15.11x_2^2x_4^2 - 52.55x_1^2x_2^2x_3^2 \\
&+ 15.29x_1^2x_2^2x_4^2 + 27.65x_1^2x_3^2x_4^2 + 24.15x_2^2x_2^2x_4^2 + 45.35x_1^2x_2^2x_3 + 33.0x_1^2x_2^2x_{34} - 9.95x_1^2x_3^2x_2 + 12.84x_1^2x_3^2x_4 \\
&- 42.67x_1^2x_3^2x_{24} - 43.04x_1^2x_4^2x_3 + 53.65x_1^2x_4^2x_{23} + 50.8x_2^2x_3^2x_4 - 33.36x_2^2x_4^2x_1 - 49.0x_2^2x_4^2x_3 - 25.42x_2^2x_4^2x_{13} \\
&+ 23.64x_3^2x_4^2x_1 - 20.64x_3^2x_4^2x_2 + 11.56x_1^2x_2^2x_3^2x_4 + 58.85x_1^2x_3^2x_4^2x_2 - 11.36x_2^2x_3^2x_4^2x_1 \qquad 33
\end{aligned}
$$

4. DISCUSSION

It was seen from analysis of variance, table 5 that only two main effects, which include pH and duration of storage with other interactions have significant influence on the ascorbic acid level on the red sample of cashew fruit juice. Comparing the calculated F-ratio individually with the appropriate critical table value F [0.05, 1, 162] = 3.864 reveals that only thirty-one coefficients on the red sample, which include

$b_1, b_2, b_1^2, b_2^2, b_1^2b_2, b_1^2b_3, b_1^2b_4, b_1^2b_{34}, b_2^2b_1, b_2^2b_3, b_2^2b_4, b_2^2b_{14}\ b_2^2b_{34},$
$b_2^2b_{134}, b_3^2b_{12}, b_3^2b_{24}, b_3^2b_{124},\ b_2^2b_3^2b_{14}, b_3^2b_4^2b_1,\ b_4^2b_2,\ b_4^2b_1, b_4^2b_{23}, b_1^2b_4^2, b_2^2b_3^2, b_2^2b_4^2,\ b_1^2b_2^2b_3^2, b_2^2b_3^2b_4^2,$
$b_1^2b_2^2b_3^2b_4^2, b_1^2b_3^2b_2^2, b_1^2,\ b_3^2b_{24}^2b_{12},$ and $b_1^2b_2^2b_4^2b_3$

were insignificant at 5 percent. The ANOVA showed that the highest effect was at the interactions of total soluble solid, pH and duration of storage ($b_2^2b_3^2b_4$), which means that the combinations of these factors strongly affect the ascorbic acid of the red sample of juice.

The analysis of variance of yellow sample of cashew fruit juice from table 6 showed that three main effects, which are temperature, pH and duration of storage with other interactions significantly, affect the ascorbic acid level of the juice. It was seen that twenty coefficients on the yellow sample

$b_2, b_1^2b_2, b_1^2b_3^*, b_1^2b_4^*, b_1^2b_{34,} b_2^2b_{34}, b_3^2b_4,$
$b_3^2b_{12}, b_3^2b_{124}^*, b_4^2b_1^*, b_4^2b_{23}, b_1^2b_3^2, b_3^{2*}b_4^2, b_1^2b_2^2b_3^2b_4^2, b_1^2b_2^2b_4, b_1^2b_4^2b_2, b_2^2b_3^2b_1, b_2^2b_3^2b_{14}, b_3^2b_4^2b_{12},$ and $b_1^2b_2^2b_4^2b_3$

were insignificant at 5 percent. The ANOVA of the yellow sample also recorded that the highest effect was at the interactions of temperature and total soluble solid ($b_1^2b_2^2$), which means that the combinations of these factors strongly affect the ascorbic acid of the juice.

However, insignificance of these effects do not necessarily imply that they were unimportant but that the response (y) was unaffected if these effects were varied over the level of consideration, that was from -1, 0 and +1 in coded units in the design.

It was seen from equation 32 that only two main effects which include pH (with coefficient b_3 = -15.96) and duration of storage (with coefficient b_4 = -18.76) with other interactions in the model have significant influence .on the level of the ascorbic acid on the red cashew fruit juice sample while three main effects in equation 33, which include temperature (with coefficient b_1 = 12.87), pH (with coefficient b_3 = -21.38) and duration of storage (with coefficient b_4 = -20.82) with other interactions in the model have significant influence on the level of the ascorbic acid on the yellow cashew fruit juice sample. These imply that high levels of each of these factors with their interactions led to drastic reduction in the ascorbic acid level of the juice.

5. CONCLUSION

The analysis of results of the experiment and the developed models showed that pH and duration of storage for red sample and temperature, pH and duration of storage for yellow sample, with other interactions, were the major parameters that govern the shelf

life and also important factors for characterizing the quality of the samples of the juice. These quality variables enabled the prediction of shelf-life of the juice under non-refrigerated storage and distribution conditions. The ANOVA from both samples of the juice showed that the interactions of total soluble solid with other factors significantly affect the ascorbic acid of the juice.

Models developed (equations 32 and 33) showed that 31 insignificant regression coefficients of red samples and 20 insignificant regression coefficients of yellow samples were recorded at 5 percent after checking the adequacy of the predicted models. The positive signs against the coefficients of the interactions in these models showed that the levels of ascorbic acids were raised by increasing the level of factors from low to intermediate and to high levels while negative signs against the coefficients of the interactions showed that the levels of ascorbic acids were reduced from low to intermediate and to high levels.

Equations 33 and 34 express the fitted models for predicting shelf life of red and yellow samples of cashew fruit juice. The statistical analysis of the experimental data showed that the samples of cashew fruits juice models were adequate for shelf life prediction. Since the models were purely for non-refrigerated storage and distribution conditions, it is recommended comparing cashew fruits juice at different location within Nigeria, using the above experimental and modeling format, to ascertain the deteriorating differences in locations as further studies.

Conflicts of Interest

None.

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